thereof in an amount effective to increase the content of omega-3 highly unsaturated fatty acid in said milk product, wherein said milk product is selected from the group consisting of milk, cheese and butter, and wherein said animal is selected from the group consisting of cows, sheep and goats.

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REMARKS

It appears that the Office Action Summary has incorrectly listed Claims 28-51 as being pending in the present application. Claims 29-51 were pending. Claims 30, 37-38, 46-47 and 51 have been amended. New Claims 52-68 have been added. Upon entry of this amendment Claims 29-68 will be pending in this patent application.

Rejection under 35 U.S.C. §112, second paragraph

Claims 30, 37-39, 46-48 and 51 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out distinctly claim the subject matter of the invention. Specifically, the Examiner alleges that the terms "the average" and "the same genus" lack antecedent basis in Claim 30, and that alternative expressions (e.g. and/or) are improper in Claims 37, 38, 46, 47 and 51. In order to overcome the Examiner's rejections under 35 U.S.C. §112, second paragraph, claims 30, 37-38, 46-47 and 51 have been amended to particularly point out and distinctly claim the subject matter of the present invention.

In view of the foregoing, it is respectfully requested that the rejections under 35 U.S.C. §112, second paragraph, be withdrawn.

Rejection under 35 U.S.C. §102 (b)

Claims 29-50 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Ise or Hagemeister et al. Specifically, the Office Action alleges that the composition of the milk product derived from the disclosure of Ise or Hagemeister *et al.* is the same as the milk product composition claimed in the present application. It is respectfully requested that this rejection be withdrawn as the fatty acid composition of the milk product of the present invention is different from the fatty acid composition of the milk product derived from the disclosure of Ise or Hagemeister *et al.*

Hagemeister *et al.* disclose feeding menhaden oil to lactating cows to allegedly increase the omega-3 fatty acid content of the milk. The Hagemeister abstract cited by the Examiner does not disclose the fatty acid profile for the milk product. Therefore, research was conducted in order to find information regarding the fatty acid profile of milk for cows which had been fed fish oil. Table 1 shows one of the results obtained regarding the fatty acid composition comparison between milk produced from cows fed with tuna oil, which has higher amount of DHA than menhaden oil, and milk produced from cows according to the present invention. The amount of DHA fed to cows in each case is about 30 g per cow per day.

Table 1. Selected fatty acid as percent of total fatty acid in milk produced from cows fed with tuna oil and microbial organisms of the present invention.

	Tuna oil		Present Invention	
Fatty acid	Control	12 days of feeding	Control	14 days of feeding
C20:1	<0.1	0.00	<0.1	0.11
C20:2	0.95	1.2	0.58	0.69
C20:4+C22:1	0.23	0.25	0.29	0.34
C20:5 n3 (EPA)	0.00	0.05	<0.1	<0.1
C22:5 n6 (DPA)	0.00	0.00	0.00	0.18
C22:6 n3 (DHA)	0.00	0.07	0.00	0.33

numbers represents % of total fatty acid.

As Table 1 clearly shows, the fatty acid content of the milk from cows fed fish oil is different from the fatty acid content of the milk from cows fed microorganisms of the present invention. The amount of DHA incorporation using the microbial organisms of the present invention is significantly higher than milk from cows fed tuna oil, 0.33 and 0.07, respectively. This may be due to the fact one of the advantages of the present invention with regard to feeding whole cell microorganisms is that the long chain fatty acids in the microorganisms are partially protected from digestion and hydrogenation in the cows rumen because they are naturally encapsulated by the cell walls of the microorganisms. This encapsulation also protects these fatty acids from oxidation in the feed prior to consumption by the cows. Thus, more net amount of DHA may be actually consumed by the cows, and more net amount of DHA may be passed through the rumen, thereby resulting in a greater increase of the amount of DHA in the milk compared to fish oil. DHA and other fatty acids in unprotected fish oil would readily

oxidize in the feed and be readily hydrogenated in the rumen, thus resulting in a lower amount of DHA content in the milk. DHA is important for brain and nervous system development and cardiovascular health in humans; therefore, a higher amount of DHA is desirable.

In addition, fish oils, such as tuna oil and menhaden oil, do not contain any significant amount of docosapentaenoic acid (DPA, 22:5 n-6, an omega-6 fatty acid) compared to the microorganisms of the present invention; therefore, as shown in the above table, the resulting milk does not contain any appreciable amount of DPA. In contrast, microbial organisms disclosed in the present invention contain DPA n-6 which results in incorporation of DPA n-6 in the milk. This represents an effective way to increase the long chain omega-6 content of the milk as it is recognized that some of the DPA can be converted to omega-6 arachidonic acid in humans. Omega-6 arachidonic acid produces eicosanoids, which facilitate growth in children, and therefore the milk product of the present invention is particularly beneficial to children because it leads to milk with a significantly improved total long chain omega-6 fatty acid content <u>and</u> DHA content.

Another set of fatty acid content data obtained for cows fed fish oil is listed in Table 2.

Table 2. Selected fatty acid as percent of total fatty acid in milk produced from cows fed with fish oil and microbial organisms of the present invention.

	Fish oil		Present Invention	
Fatty acid	Feeding protocol #1	Feeding protocol #2	Control	14 days of feeding
C20:1	0.38	0.21	<0.1	0.11
C20:2	0.21	0.26	0.58	0.69
C20:4+C22:1	0.11	0.23	0.29	0.33
C20:5 n3 (EPA)	0.39	0.31	<0.1	<0.1
C22:5 n6 (DPA)	N.D.	0.05	0.00	0.18
C22:6 n3 (DHA)	0.23	0.39	0.00	0.33

numbers represents % of total fatty acid.
N.D. = not detected

Although the amount or the time of feeding is not provided for the two fish oil feeding protocols, based on the amount of DHA present in the milk, it is believed that feeding protocol #2 was for a longer feeding period. As Table 2 shows, even if the milk from fish oil fed cows contains similar amount of DHA, the amount of total long chain omega-6 fatty acids (C20:4 n6+C22:5 n6) present in the milk is significantly lower. Thus, when the amount of fish oil fed to a milk-producing animal is adjusted to provide the milk having similar DHA fatty acid content, the total long chain omega-6 fatty acid content, *e.g.*, DPA n6 + ARA n6, will not be similar or equal to that of the milk of the present invention. Therefore, it is not possible to produce the same or similar fatty acid composition as in milk of the present invention by feeding fish oil to milk-producing animals.

Importantly, the milk from cows fed fish oil has a different taste and odor than the milk from cows fed microorganisms of the

present invention. *See for example*, Lacasse, *et al.*, *J. Anim. Sci.* Vol. 76, Suppl. 1/*J. Dairy Sci.*, Vol 81, Suppl. 1/1998, page 231, abstract number 901 (copy attached). This difference in taste and odor is most likely due to the presence of certain compound(s) in the milk from cows fed fish oil which are absent in the milk from cows fed microorganisms of the present invention. Therefore, it is evident that the milk produced by feeding fish oil and the milk produced by feeding microbial organisms of the present invention is different. In fact, in some cases feeding fish oil to lactating cows "negatively affected taste of milk." *Id.* In contrast, there is no "fishy" odor or taste present in the milk product of the present invention.

Ise discloses feeding an omega-3 fatty acid source in conjunction with vitamin E and water which contains silicic acid, glucanase, cellulase, calcium and phosphorus. See, for example, abstract, Claim 1, Col. 6, lines 65-66, and Col. 7, lines 3-15. Similar to Hagemeister *et al.*, Ise uses fish oil, particularly menhaden oil. See Col. 5, lines 54-57. Therefore, the fatty acid composition of the milk product derived from the teachings of Ise will be different from the fatty acid composition of the milk product derived from the present invention for the reasons stated above.

Even assuming, *arguendo*, that Ise feeds only a "pure" omega-3 fatty acid (it is maintained that no such teaching is present in the disclosure by Ise), it is evident that the fatty acid composition of the milk products is different. For example, a "pure" omega-3 fatty acid lacks DPA, an omega-6 fatty acid.

Therefore, unlike the milk product of the present invention, the milk product derived from feeding a pure omega-3 fatty acid will not have an increased DPA content.

In view of the foregoing, withdrawal of 35 U.S.C. §102(b) rejection is respectfully requested.

Rejection under 35 U.S.C. §103(a)

Claims 29-50 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over either one of Hagemeister *et al.* or Ise taken together with either one of Ellenbogen *et al.* or Long. The Examiner admits that both Hagemeister et al and Ise are silent concerning the omega fatty acid source, coating the feed supplement with protein to protect it from degradation in the rumen, and using dry grain which has been prepared by extrusion. However, it appears that the Examiner is asserting it would have been obvious to one of ordinary skill in the art to combine the teaching of Hagemeister *et al.* and/or Ise with the teaching of Ellenbogen *et al.* or Long. The Examiner further asserts that encapsulating feed supplement and pelletizing dry grain feed are well known in the art. Reconsideration of this rejection is respectfully requested as the combination of the teaching of Hagemeister *et al.* and/or Ise with the teaching of Ellenbogen *et al.* or Long is improper because there is no suggestion whatsoever for making the combination.

As will be recognized, claims cannot be found obvious in view of a combination of references unless the prior art itself suggests the desirability of the combination. *Berghauser v. Dann,* 204 U.S.P.Q. 393 (D.D.C. 1979); *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 221 U.S.P.Q. 929 (Fed. Cir. 1984). There must

be something in the prior art that would have motivated persons of ordinary skill to make the combination. *In re Stencel*, 4 U.S.P.Q.2d 1071, 1073 (Fed. Cir. 1987), *accord*, *Ex parte Marinaccio*, 10 U.S.P.Q.2d 1716 (Pat. Off. Bd. App. 1989) (combining references is improper absent some teaching, suggestion, or motivation for the combination in the prior art). In this respect, the following statement by the Patent Office Board of Appeals is noteworthy:

> Our reviewing courts have often advised the Patent and Trademark Office that it can satisfy the burden of establishing a *prima facie* case of obviousness only by showing some objective teaching in either the prior art, or knowledge generally available to one of ordinary skill in the art, that "would lead" that individual "to combine the relevant teachings of the references." ... Accordingly, an examiner cannot establish obviousness by locating references which describe various aspects of a patent applicant's invention without also providing evidence of the motivating force that would **impel** one skilled in the art to do what the patent applicant has done.

In re Levengood, 28 U.S.P.Q.2d 1300, 1302 (Pat. Off. Bd. App. 1993) (citations omitted; emphasis added). Significantly, the Office Action identifies no "motivating force" that would "impel" persons of ordinary skill to combine the respective teachings of the cited references in a manner that would produce the claimed inventions.

The present invention is directed to a milk product which is derived from an animal that is raised by feeding a feed material which contain omega-3 highly unsaturated fatty acids from cultured microbial organisms to increase the amount of omega-3 highly unsaturated fatty acid in the corresponding milk product.

For the sake of brevity, the above discussions regarding the teachings of Hagemeister *et al.* and Ise are incorporated herein by

reference in their entirety. It appears that Ellenbogen *et al.* simply lists the type of polyunsaturated acids which are produced by marine fungi, and Long discloses a method of manufacturing omega-3 fatty acids by heterotrophically culturing Thraustochytrids. Neither Ellenbogen *et al.* nor Long teach or suggest using marine microorganisms as a feed material for milk-producing animals to increase the omega-3 content of the milk. Although Long has a very brief mention of using <u>extracted</u> lipids from microorganisms as a feed additive for animals, the Long reference does not recognize or suggest that such additives would be useful in increasing the omega-3 fatty acid content of the animal. Long does not disclose or suggest using whole cell microorganisms for feeding animals of any type or using the whole cell microorganisms or extracted oils in the feeding of milk-producing animals. Long does not disclose or suggest that milk would have an increased omega-3 fatty acid content. Long does not teach or suggest increasing the DPA, an omega-6 fatty acid. Furthermore, the very brief disclosure of Long regarding the addition of the <u>extracted</u> oils to animal feed includes no recognition of downstream benefits to those consuming the animal. More importantly, there is no recognition, teaching or suggestion by Long to change the fatty acid content of milk products by feeding the whole cell microorganism or extracted oil to milk-producing animals; therefore, Long does not impel one of ordinary skill in the art to produce a milk product with increased omega-3 fatty acid content by feeding whole cell microorganisms to a milk-producing animal. Since there is no disclosure or suggestion that

would impel one skilled in the art to combine the teachings of Long or Ellenbogen *et al.* with Ise or Hagemeister *et al.*, the Examiner's rejection under 35 U.S.C. §103(a) based on the combined references of Long or Ellenbogen *et al.* with Ise or Hagemeister *et al.* is improper.

The Office Action points to nothing in the cited references that would "impel" preparation of a milk product that is derived from an animal that was raised on a feed material containing cultured microbial organisms containing omega-3 highly unsaturated fatty acid. The Office Action alleges that both Hagemeister *et al.* and Ise are silent concerning the omega fatty acid source. But as the abstract of Hagemeister *et al.* and the disclosure of Ise, as discussed above, clearly indicates that the source of omega-3 fatty acid is fish oil, in particular menhaden oil.

What the Office Action appears to suggest is that the claimed milk product would have been obvious because it would have been **possible** to change the source of omega-3 fatty acid as disclosed in Hagemeister *et al.* or Ise with the sources disclosed by Ellenbogen *et al.* or Long. However, none of the teachings of Hagemeister *et al.*, Ise, Ellenbogen *et al.* and Long even contemplate producing milk product using microorganisms as a source of feed. The mere **possibility** that the prior art can be combined and modified does not itself provide the requisite motivation to do so. *In re Dien*, 152 U.S.P.Q. 550 (C.C.P.A. 1967) (incentive to seek improvement of existing process held to not render change made by applicant obvious, even where the change was one capable of being made from theoretical point of view). It is only with the improper use of

hindsight and with the benefit of the disclosure of the present application that one can discern the desirability of the particular milk product now claimed.

Without showing a "motivating force that would **impel** one skilled in the art to do what the patent applicant has done," which is neither taught nor suggested in the prior art, the Office Action merely suggests that it would have been possible or "obvious to try." However, "obvious to try" is not the standard of 35 U.S.C. §103. *In re Geiger*, 2 U.S.P.Q.2d 1276, 1278 (Fed. Cir. 1987).

In view of the foregoing arguments, it is respectfully requested that the rejection under 35 U.S.C. §103 be withdrawn.

It is not admitted nor acknowledged that the cited references are prior art, and the right to swear behind these references is expressly reserved.

Respectfully submitted,

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898 Plasma leptin concentrations and first postpartum ovulation in dairy cows differing in energy balance.

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This study examined leptin concentrations in dairy cows differing widely in energy balance (EB) during the early postpartum (PP) period. Multiparous Holstein cows (n=32) were assigned to one of 3 energy balance groups consisting of NLAC cows (n=8, not milked after calving), LAC2 cows (n=13, milked 2x/day) and LAC3 cows (n=11, milked 3x/day). LAC3 cows were fed a less energy dense ration. Daily EB was calculated for all cows and ovarian follicular development monitored by ultrasonography. Blood was collected daily for analysis of leptin (Linco Research). Body weight (BW) and body condition score (BCS) were monitored weekly. Energy balance (P<0.001), BW loss (−5 ± 22, −25 ± 22 and −48 ± 27 kg, P<0.004) and BCS loss (+0.1 ± 0.2, −0.6 ± 0.4 and −0.7 ± 0.4, P<0.001) for NLAC, LAC2 and LAC3, respectively, were significantly different among groups during the first 3 weeks PP. Days to first ovulation PP did not differ (P=0.15) among EB groups (18 ± 5, 29 ± 4 and 31 ± 4 for NLAC, LAC2 and LAC3, respectively. The dominant follicle (DF) from the first follicular wave PP ovulated in 75% (6/8) of NLAC, 54% (8/13) of LAC2 and 36% (4/11) of LAC3 cows (P>.2). Leptin concentrations were similar (P>0.9) among EB groups from days 5 to 21 PP (4.5 ± 2.3, 4.3 ± 2.2 and 4.1 ± 1.8 ng/ml for NLAC, LAC2 and LAC3, respectively). A small, but significant increase in leptin concentrations was observed from day 5 (3.9 ± 1.9 ng/ml) to day 21 (4.4 ± 2.2 ng/ml, P<0.01). Leptin levels for cows that lost less than or more than the mean BCS loss (−0.5 ± 0.5) were 4.9 ± 2.7 and 3.7 ± 1.1 ng/ml, respectively (P=0.13). There was a trend (P<0.06) for cows that ovulated the first DF to have higher concentrations of leptin (4.8 ± 2.3 ng/ml) compared to non-ovulating cows (3.5 ± 0.7 ng/ml). In summary, leptin concentrations did not differ between NLAC, LAC2 and LAC3 cows during days 5 to 21 PP, despite significant differences in EB, BCS and BW during this period. In PP cows, higher leptin concentrations may contribute to ovulation of the first DF.

Key Words: Leptin, Follicle, Cows

899 Serum leptin is associated with carcass traits in finishing cattle.

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Leptin, a recently discovered hormone of adipose tissue origin, is associated with measures of body fat content in rodents and humans. The major physiological action of leptin is that it exerts a negative effect on food intake, although it also increases metabolic rate. We hypothesized that cattle in the final phase of the finishing period might have increased leptin in circulation as ribeye fat thickness and intramuscular fat (marbling) also increased. Although leptin mRNA was found in bovine adipose tissue, to date, no quantitative assay was available to measure circulating leptin in cattle. We validated a commercially available leptin assay (Linco Research, Inc.; cat. XL-85K) for use in bovine serum. Human leptin was used as standard in the assay. Varying volumes of a pooled sample of bovine serum produced a binding curve that paralleled the standard curve. When concentration of leptin measured in the assay from bovine serum was regressed on volume assayed, the 95% confidence interval (CI) about the slope of the regression line included 0. Leptin (human) also could be quantitatively recovered from bovine serum. Regression of leptin concentration measured by the assay on concentration expected in bovine serum samples to which leptin had been added produced a regression line with a slope and CI that included 1.0. The assay was sensitive to 1 ng/ml human leptin equivalent. This assay was used to measure leptin in serum from finishing heifers. In brief, these animals were part of a 120-d finishing study designed to evaluate the effect of added dietary choline and fat on growth performance and carcass traits. Samples of serum were obtained from all animals on the study (n=318) 30 d prior to slaughter for leptin determination. At slaughter, ribeye fat thickness, marbling score, yield grade, and kidney, pelvic, and heart fat (KPH) were determined. Leptin ranged from 2.8 to 15.3 ng/ml. Serum leptin was positively correlated (P<.001) with ribeye fat thickness (r=.32), KPH (r=.18), marbling score (r=.28), and yield grade (r=.28). The data suggest that circulating leptin is positively associated with measures of carcass fatness in cattle.

Key Words: Leptin, Carcass Traits, Cattle

900 Feeding protected and unprotected fish oil to dairy cows: I Effect on animal performances.

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Thirty Holstein cows in mid-lactation (158 ± 20 days in lactation) were fed with a total mix ration based on haylage, corn silage, high moisture corn grain and a supplement. Cows were paired into 4 groups, according to the DMI and milk yield. After one preliminary week, cows' ration have been mix with nothing (control), unprotected "dry" fish oil (UFO; 4% of DM; Vaculift, Ca) or glutheraldehyde-protected microcapsules of fish oil (PFO; Ocean Nutrition Ltd, NS), at 2% or 4 % of DM, for four weeks. The UFO and PFO supplements contained 42 an 40% of lipid; of which, 28.7 % and 25.5 % were polyunsaturated fatty acids. Cows fed with UFO reduced their feed intake by more than 25 % (P<0.01). Consequently, UFO fed cows loose body weight (P<0.01) and reduced their milk production (P<0.01). Feed intake, milk production and ADG were unaffected by feeding PFO at 2 or 4% (P>0.1). Feeding UFO or PFO reduced (P<0.01) milk fat content. During the treatment period, milk fat content averaged 3.14, 2.41, 2.18 and 2.47 for control, 2%PFO, 4%PFO and UFO, respectively. Milk protein content was lower in cows fed UFO (3.16%; P<0.01), 4%PFO (3,15%; P<0.01) or 2%PFO (3.25%; P<0.1) than in cows fed the control diet (3.39%). Feeding unprotected fish oil to lactating dairy cows reduces animal performances. Fish oil, unprotected or protected from ruminal biohydrogenation by glutheraldehyde treatment, reduces protein and fat content of milk. Project supported by Ralston Purina International.

Key Words: Milk Fat, Fish Oil

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41:74 + 236

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901 Feeding protected and unprotected fish oil to dairy cows: II Effect on milk fat composition.

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Thirty Holstein cows in mid-lactation were fed with a total mix ration (control), supplemented with unprotected "dry" fish oil (UFO; 4% of DM) or glutheraldehyde-protected microcapsules of fish oil (PFO) at 2% or 4 % of DM, for four weeks. The UFO and PFO supplements contained 42 an 40% of lipid; of which, 28.7 % and 25.5 % were polyunsaturated fatty acids. Feeding fish oil decreased (P<0.01) the proportion of short chain fatty acids (less than 14 carbons) in milk fat. At the end of the treatment period, these fatty acids represented 11.7, 10, 7.6 and 8.3 % of the total fatty acids for control, 2%PFO, 4%PFO and UFO, respectively. Similarly, the proportion of stearic and oleic acids were reduced (P<0.01) in fish oil fed cows. Milk fat trans C:18:1 fatty acids at the end of the experiment was 2.9% for control cows, but, increased in both UFO (9.7%; P<0.01) and 4%PFO (8%; P<0.01). Therefore, it is likely that the protection against rumen biohydrogenation was incomplete. Content of very long chain fatty acids, including arachidonic acid (C20:4), eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6) increased (P<0.01) with fish oil feeding. Polyunsaturated fatty acids (PUFA) content was higher in 2%PFO (3.8%; P<0.1), 4%PFO (4.2%; P<0.01) and UFO (5.7%; P<0.01) than in control (3.0%). Accordingly, the peroxide index increased (P<0.05) and a taste panel was able to detect unusual taste in 4%PFO milk and disliked UFO milk. In conclusion, feeding fish oil to lactating cows decreased de novo synthesis of fatty acids, increased long chain PUFA and susceptibility of milk to oxidation and negatively affected taste of milk. Project supported by Ralston Purina International and Alberta Agriculture Research Institute.

Key Words: Milk Fat, Fish Oil, Fatty Acids